(UNDATED)

DATA EVALUATION RECORD

AMINE OXIDE

Study Type: 82-1a; Subchronic Oral Toxicity Study in Rats

Work Assignment No. 3-27 (MRID 44475203)

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: 13-Week subchronic toxicity [feeding]- rat
OPPTS Number: 870.3100 OPP Guideline Number: §82-1a

DP BARCODE: D244775 SUBMISSION CODE: S538885
P.C. CODE: 000439

TEST MATERIAL (PURITY): Amine oxide (27.72% a.i.)

SYNONYMS: P0434

CITATION: Cardin, C.W., D.H. Pence, D.A. Banas, R.D. Alsaker, and K.S. Greenspun. (1978) Thirteen-week subchronic dietary administration to male and female rats - P0434. Hazleton Laboratories, Inc., Vienna, VA. Project No. 297-329. October 4, 1978. MRID 44475203. Unpublished.

SPONSOR: The Proctor & Gamble Company, Cincinnati, Ohio 45202.

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 44475203), amine oxide (27.72% a.i.) was administered to Sprague-Dawley rats (20/sex/dose) by feeding at dose levels of 0, 0.1, 0.2, or 0.4% (0, 63, 112, or 236 mg/kg/day for males; 0, 80, 150, or 301 mg/kg/day for females) for 13 weeks.

In the 0.4% treatment groups at 13 weeks, body weight gains of male and female rats were 23-26% lower and alkaline phosphatase activities were 28-60% higher than the controls. In addition, 12/20 males and 8/20 females had lenticular opacities (cataracts). In the 0.2% treatment groups at 13 weeks, body weight gains were 8 and 18% lower in males and females, respectively, alkaline phosphatase activities were 45% higher in males, and 3/19 males had cataracts compared to the controls. Reduced food consumption (≤8% during the final 4 weeks) was observed in both the 0.4 and 0.2% treatment groups. No treatment-related deaths occurred during the study. There were no biologically significant differences between rats in the 0.1% treatment groups and the controls. There were no treatment-related clinical signs or differences in the hematology, urine, organ weights, or gross or microscopic histology of the treated

rats compared to the controls. No neoplastic tissue was observed. **The LOEL for this study in 0.2% (112 mg/kg/day), based on decreased body weights in females and the development of cataracts in males. The NOEL is 0.1% (63 mg/kg/day).**

This subchronic toxicity study in rats is classified **acceptable (§82-1a)** and satisfies the Subdivision F guideline requirement for a subchronic toxicity study in rodents. No life-threatening responses appeared to result from the administration of amine oxide at the highest dose level (0.4%; 301 mg/kg/day). Therefore, longer-term studies should be conducted at or above this treatment level.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided. A Flagging statment was not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Amine oxide
   Description: Clear, colorless liquid
   Lot: Not reported
   Purity: 27.72% a.i.
   Stability of compound: Not reported
   CAS #: 70592-80-2
   Structure: Not provided

2. Vehicle and/or positive control: None

3. Test animals: Species: Rat
   Strain: Sprague-Dawley
   Age and weight at study initiation: 6 weeks of age; body weight range - males, 167.6-231.1 g, females, 124.2-173.5 g
   Source: Charles River Breeding Laboratories, Inc., Wilmington, MA
   Housing: Individually housed in elevated stainless steel wire mesh cages
   Diet: NIH-07 powered diet (Ziegler Brothers, Inc., Gardners, PA), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions: Not provided
   Acclimation period: 2 Weeks

B. STUDY DESIGN

1. In life dates - Start: 6/29/78 End: 9/28/78

2. Animal assignment

   One week after arrival, animals of questionable health or outlying body weights (±15%) were discarded. Prior to initiation of the study, the remaining rats were randomized and assigned to the test groups in Table 1.

Table 1: Study design.[a]

Test Group	Nominal concentration in feed (%)	Dose to animal[b] (mg/kg/day)		Animals assigned	
		Male	Female	Male	Female
1 Control	0	0	0	20	20
2 Low	0.1	63	80	20	20
3 Mid	0.2	112	150	20	20
4 High	0.4	236	301	20	20

[a] Justification for the dose levels was not provided.
[b] Dose to animal was calculated by the reviewer based on the mean measured concentration in the feed and the mean body weights and food consumption for the entire 13-week treatment period for each treatment group.

3. Treatment preparation

The test diets were prepared fresh weekly. Sufficient amine oxide for each treatment rate was added to 2 kg of untreated diet and mixed for approximately 5 minutes in a Hobard mixer. The treated diet was sifted using a fine mesh screen, ground into a fine powder, and mixed for 1 minute per kg in a Twin-Shell blender equipped with an intensifier bar. (It was stated that this method was not used to prepare the diet for Week 1. The alternate method was not described.) The ground treated diet was stored at 40 ± 4 F in seal-tight plastic buckets during Week 1 and in glass jars during Weeks 2-13.

To determine homogeneity, the top, middle, and bottom strata of diets prepared prior to the initiation of the study and of diet prepared for weeks 1, 2, and 4 were collected for analysis. To determine stability, diet prepared prior to the initiation of the study was stored at 40 ± 4 F for up to 12 weeks. To determine concentration, samples were collected from all freshly prepared diets throughout the study for random analysis.

Results:
Homogeneity[a]:
0.1%: 0.09-0.2%
0.2%: 0.18-0.33%
0.4%: 0.24-0.46%

Stability analysis[a]:
0.1%:
Week 0: 0.18%
Weeks 1-12: 0.13-0.22%
Week 13: 0.21%
0.2%:
Week 0: 0.25%
Weeks 1-12: 0.19-0.36%
Week 13: 0.28%
0.4%:
Week 0: 0.41%
Weeks 1-12: 0.37-0.49%
Week 13: 0.44%

[a] Data are not adjusted for background.

Concentration analysis:
0.1%: 0.11 ± 0.03%
0.2%: 0.20 ± 0.04%
0.4%: 0.37 ± 0.03%

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics

Statistical analysis using both ANOVA and distribution-free techniques were performed on numerical parameters. If Bartlett's test of homogeneity of variance was not significant, comparisons with the control were based on the least significant difference criterion. If Bartlett's test was significant, Wilcoxon's rank sum test was used. Regression analysis using dose as the independent variable was performed. All statistical tests were based upon a 5%, two-sided risk level.

C. METHODS

1. Observations

All rats were observed for mortality and moribundity in the morning and afternoon of weekdays and "twice daily" on weekends, with the exception of the first two weekends when they were observed only once daily. Observations of gross signs of toxicity and pharmacologic effects were recorded weekly.

2. Body weight

Body weights of all rats were measured during acclimation (Week -1), immediately prior to the initiation of treatment, once each week during treatment and immediately prior to sacrifice.

3. Food consumption

Food consumption for each animal was measured weekly during treatment, and was reported as g/animal/week. It did not appear that a distinction was made between food consumption and food wastage. Food efficiency was calculated on a weekly basis throughout the study.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were conducted on all rats prior to the initiation of treatment and during Weeks 6 and 12. Testing was done with a hand-held biomicroscope and indirect ophthalmoscope following administration of a mydriatic solution.

5. Blood

Blood was collected from all surviving animals at Week 7 by segmental tail amputation and from the abdominal aorta at sacrifice. The rats were fasted prior to blood collection. The CHECKED (X) parameters were examined in all samples analyzed.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Partial thromboplastin time)		
	(Capillary clotting time)		
	(Prothrombin time)		

* Required for subchronic toxicity studies.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
	Calcium*		Albumin*
	Chloride*		Bilirubin (total)
	Magnesium		Blood creatinine*
	Phosphorus*	X	Blood urea*
	Potassium*		Cholesterol
	Sodium*		Globulin
		X	Glucose* (fasting)
			Total serum protein*
	ENZYMES		Triglycerides
X	Alkaline phosphatase		
	Cholinesterase		
	Creatine phosphokinase		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase		
X	Serum aspartate aminotransferase		
	Glutamate dehydrogenase		

* Required for subchronic toxicity studies.

6. Urinalysis*

Urine was collected from all surviving animals during Weeks 7 and 13 of treatment. Urine was collected every 30 minutes for a period of 6 hours until a sufficient quantity was collected to perform the analyses. Animals were individually housed in metabolism cages during the urine collection period. The CHECKED (X) parameters were examined in all samples analyzed.

X	Appearance	X	Glucose
	Volume		Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrite
X	Albumin		Urobilinogen

* Urinalysis is not required for subchronic toxicity studies.

7. Sacrifice and Pathology

Animals were euthanized by exsanguination under sodium pentobarbital anesthesia during Week 14. The bodies were subjected to gross pathological examination. The CHECKED (X) tissues were collected from the main group animals for histological examination. Histological exams were conducted on all tissues from the control group rats, from

all females in the 0.2% group, and from 10 rats/sex in the 0.4% group. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Sciatic nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord*
X	Stomach*		(femur)	X	Pituitary*
X	Duodenum*	X	Lymph nodes*	X	Eyes*
X	Jejunum*	X	Spleen*		Optic nerve
X	Ileum*	X	Thymus*		
X	Cecum*				
X	Colon*		UROGENITAL		GLANDULAR
X	Rectum*				
XX	Liver*	XX	Kidneys*	X	Adrenal gland*
	Gall bladder*	X	Urinary bladder*		Lacrimal gland
X	Pancreas*	XX	Testes*	X	Mammary gland
			with epididymides	X	Thyroids*
		X	Prostate	X	Parathyroids*
		X	Seminal vesicle		
	RESPIRATORY	XX	Ovaries*		
		X	Uterus*		
X	Trachea*		Vagina		
X	Lungs with				
	bronchi*				
	Muzzle				OTHER
	Pharynx				
X	Larynx			X	Bone*
					(femur)
				X	Muscle*
					(thigh)
				X	Costochondral
					junction
				X	Skin*
				X	All gross lesions
					and masses*

* Required for subchronic toxicity studies.

II. RESULTS

A. Observations

1. Mortality - There were no treatment-related deaths during the study. One male rat in the 0.2% treatment group died of unrelated causes during Week 9.

2. Clinical Signs - No treatment-related changes were observed in the appearance or behavior of the rats during the study.

B. Body weight and weight gain

Body weights were lower ($p<0.05$) for males and females in the 0.4% treatment group throughout the study, and for females in the 0.2% treatment group during Weeks 3-5 and 9-13 compared to the controls. Body weight gains of male and female rats in the 0.4% treatment groups were 23-26% lower ($p<0.05$) than the controls at the termination of treatment (Table 2). Body weights and body weight gains of male and female rats in the 0.2% treatment group were 8 and 18% lower, respectively, than the controls. Male and female rats in the 0.1% treatment group were unaffected by treatment.

Table 2. Mean body weights and body weight gains (g) of rats during oral (feeding) treatment with amine oxide.[a]

Treatment rate (%)	Body Weight (g)			Body weight gain (g)	
	0 Weeks	4 Weeks	13 Weeks	Total (g)	% Control gain
Males					
0	206	365	494	288	---
0.1	208	361	481	273	-5
0.2	203	352	469	266	-8
0.4	206	323*	427*	221*	-23
Females					
0	147	219	272	125	---
0.1	153	222	276	123	-2
0.2	146	206*	249*	103	-18
0.4	149	200*	242*	93*	-26

[a] Data obtained from Table 1, pages 36-37, and page 228 in the study report.
* Significantly different from the controls, $p<0.05$.

C. Food consumption and compound intake

1. Food consumption - Rats in the 0.4% treatment group wasted food during Weeks 1 (6 males, 5 females), 2 (6 males, 2 females), 3 (2 males, 1 female), 4 (2 females, and 5 (1 male, 1 female). Only rarely did wastage occur

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by rats in the control and other treatment groups. Mean food consumption by rats in the 0.2 and 0.4% treatment groups during the final 4-week period of treatment was 4-8% lower than the controls. The validity of the food consumption data is uncertain for the first part of the study when food wastage was common in the 0.4% treatment group, since no distinction was made between food that was consumed and food that was wasted. Mean feed efficiency was lower for rats in the 0.4% treatment groups at most sampling intervals compared to the controls.

2. Compound intake - Calculated compound consumption by male rats in the 0.1, 0.2, and 0.4% treatment groups averaged 63, 112, and 236 mg/kg/day, respectively, over the 13-week treatment period. Measured compound consumption by female rats in the 0.1, 0.2, and 0.4% treatment groups averaged 80, 150, and 301 mg/kg/day, respectively.

D. Ophthalmoscopic examination

At Week 13, lenticular opacities (cataracts) were found in 12/20 males and 8/20 females in the 0.4% treatment groups and 3/19 males in the 0.2% treatment groups. Indications of these conditions were noted in some rats during the Week 6 examination. No lenticular opacities were noted in the 0.1% or control groups.

E. Blood work

1. Hematology - No treatment-related differences in hematological parameters were observed.

2. Clinical Chemistry - Alkaline phosphatase activities were higher in males in all treatment groups and females in the 0.4 and 0.2% treatment groups at 7 and 13 weeks (Table 3). The difference was significant ($p<0.05$) for all male groups and the female 0.4% group at both intervals. At 13 weeks, alkaline phosphatase activities were 28-45% higher in male treatment groups and 59% higher in the female 0.4% treatment group.

All other clinical blood chemistry parameters for rats in the treated and control groups remained within the expected ranges, and observed differences did not appear to be either treatment-related or biologically significant.

Table 3. Alkaline phosphatase levels in rats following 7 and 13 weeks of treatment with amine oxide.[a]

Treatment rate (%)	Alkaline phosphatase (IU/L)	
	Week 7	Week 13
Males		
0	87.10	66.70
0.1	120.90*	91.50*
0.2	140.15*	96.47*
0.4	121.75*	85.25*
Females		
0	53.55	39.16
0.1	49.35	35.30
0.2	73.00*	46.55
0.4	94.05*	62.40*

[a] Data obtained from Table 3, pages 43 and 229 in the study report.
* Significantly different from the control, $p<0.05$.

F. Urinalysis

No treatment-related differences in urine parameters were observed.

G. Sacrifice and Pathology

1. Organ weight - Males in the 0.4% treatment group had absolute mean heart weights 10% lower than the controls, and males in the 0.2 and 0.4% treatment groups had absolute mean liver weights 11-12% lower than the controls. Relative mean heart, kidney, and brain weights were increased in the 0.2 and/or 0.4% male and female treatment groups. Relative mean testes weights were increased in the 0.2 and 0.4% male treatment groups. All organ weight differences could be attributed to reduced body weights of these animals.

2. Gross pathology - No treatment-related gross postmortem differences were observed between rats in the treated and the control groups. All abnormalities appeared to occur randomly and sporadically in all study groups.

3. Microscopic pathology

a) Non-neoplastic - No treatment-related microscopic postmortem differences were observed between rats in the

treated and the control groups. All abnormalities appeared to occur randomly and sporadically in all study groups.

b) Neoplastic - No neoplastic tissue was observed in rats in the treatment and control groups.

III. DISCUSSION

A. Investigator's Conclusions

The study authors did not identify LOEL or NOEL values or draw conclusions from this study. Based on the study summary, the study authors concluded that administration of amine oxide resulted in reduced body weights and increased blood alkaline phosphatase activities in males in all treatment groups and females in the 0.4 and 0.2% treatment groups.

B. Reviewer's Discussion

We disagree with the study author that amine oxide caused toxicologically significant effects in the 0.1% treatment group. Body weights were reduced only 5% in males and 2% in females. Although alkaline phosphatase activities were increased in approximately 40% in males in this treatment group, the degree of increase was similar to that observed in males in the 0.4% treatment group and the activities remained within the expected biological range. No other differences were observed between the 0.1% treatment groups and the controls.

Body weight gains of males and female rats in the 0.2% treatment groups were 8 and 18% lower than the controls, respectively, at 13 weeks and food consumption was reduced (<8%). Alkaline phosphatase activities were 36-61% higher in males and females at 7 weeks and in males at 13 weeks compared to the controls. Three male rats were found to have developed cataracts. No morphological or histopathological changes were observed in association with the increased activities. No other differences related to treatment were observed between the 0.2% treatment groups and the controls.

Body weight gains of male and female rats in the 0.4% treatment groups were 23-26% lower than the controls at 13 weeks, and food consumption was somewhat (<8%) reduced. The

difference in body weight gains was greater (26-29%) during the first 4 weeks of the study than during the final 9 weeks (19-20%), which may indicate that part of the reduction in body weight was due to an aversion to the food. Wastage of food was observed during the early part of the study, and food consumption data for early intervals is of uncertain validity since food wastage may not have been factored into the evaluation. Alkaline phosphatase activities were 28-40% higher in males and 60-76% higher in females at 7 and 13 weeks compared to the controls. Twelve male rats and eight female rats had developed cataracts. No other differences related to treatment were observed between the 0.4% treatment groups and the controls.

Therefore, the LOEL for this study is 0.2% (112 mg/kg/day) based on reduced body weights in females and the development of cataracts in males. The NOEL is 0.1% (63 mg/kg/day). The maximum dose rate for a longer term study should be at or above 0.4% (301 mg/kg/day).

IV. STUDY DEFICIENCIES

Several guideline deficiencies were noted in this study. The test substance was not completely characterized. Animal care was not completely described. Hematological and clinical blood chemistry analyses were incomplete. This study dates from 1978, when submission of this information was not required, and it is unlikely that the missing information is available to fill data gaps. Blood samples would no longer exist for additional analyses. Also, the results of the statistical analyses, which are presented in the tables in Attachment V, are illegible, so that all information about statistical significance had to be drawn from the text.

However, the study meets its stated purpose and the intent of the guideline requirement, which is establishing the maximum dose that should be tolerated through a 2-year chronic feeding study. The study is judged to be acceptable.

Sign-off date: 8/6/98
Sign-off branch: RASSB